International Journal of Agriculture and Biosciences 2024 14(1): 40-49.
https://doi.org/10.47278/journal.ijab/2025.008

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Lactococcus lactis spp. *lactis* a Promising Tool in the Control of Biogenic Amines during Soy Sauce Fermentation

Bhakti Etza Setiani [1,2*], Yunianta [3], Elok Zubaidah [3], Agustin Krisna Wardani [3] and Lutfi Purwitasari

[1]Doctoral Program at Faculty of Agriculture Technology, Brawijaya University, Indonesia
[2]Universitas Diponegoro, Indonesia
[3]Universitas Brawijaya, Indonesia
*Corresponding author: bhaktietza@student.ub.ac.id

ABSTRACT

Soy sauce is a fermented soybean product popular in Indonesia and is usually used as a seasoning or complement to the taste of dishes. The production process of soy sauce that is less hygienic and controlled, especially in the traditional fermentation process, can lead to the potential for excessive biogenic amines. Biogenic amines formed during the fermentation process can indicate the quality and safety of fermented products because their presence can indicate the potential for spoilage or unhygienic conditions. Additionally, excessive concentrations of biogenic amines can pose a health risk. Biological interventions to reduce biogenic amine compounds in the soy sauce production process by applying indigenous cultures of salt-resistant Lactic Acid Bacteria (LAB) can help reduce biogenic amine compounds in soy sauce. This research is expected to evaluate the content of biogenic amines in soy sauce and provide a solution to reduce the biogenic amine compounds through controlled fermentation with LAB isolates isolated from moromi stage fermentation. The results obtained from this research were soy sauce samples showed relatively high levels of putrescine and spermine. Two isolates were obtained and identified as *Lactococcus lactis*, which showed a phylogenetic relationship with *Lactococcus lactis* strain CAU3045. These isolates showed the highest capacity to degrade putrescine for 31.28% and 33.76%. Isolates FC2KB1 and FC2KB3 have shown significant potential as an alternative method to reduce biogenic amine content in soy sauce.

Keywords: Biogenic amine, Lactic acid bacteria, *Lactococcus lactis*, Moromi, Soy sauce

Article History
Article # 24-688
Received: 29-Jun-24
Revised: 01-Aug-24
Accepted: 06-Aug-24
Online First: 08-Nov-24

INTRODUCTION

Sweet soy sauce originates from Indonesia and is made from black soybeans, resulting in a thick solution with a distinctive aroma and a savory, sweet taste (Harmayani et al., 2017). Soy sauce, a basic condiment in global cuisine, derives its unique flavor and aroma from the complex blend of compounds formed during fermentation. In the fermentation process, biogenic amines (BA) have emerged as one of the determinants of soy sauce quality. Biogenic amines, including histamine, spermine, spermidine, serotonin, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, and agmatine, are natural components in soy sauce that serve as safety and quality indicators (Li et al., 2019). Soybeans contain various amino acids, and microorganisms that play an important role during the fermentation of soybean products. Specific strains of microorganisms possess enzymes known as decarboxylases, which catalyse the decarboxylation of amino acids and lead to the formation of biogenic amines (Mah et al., 2019).

Elevated concentrations of biogenic amines, especially in fermented products, have significant implications, indicating product spoilage, use of substandard raw materials, or lapses in manufacturing practices (Ekici & Omer, 2020). The Food and Drug Administration (FDA) has for a threshold for histamine level of 50mg/kg for human health. The European Food Safety Authority (EFSA) defines histamine (50mg per adult per meal), tyrosine (600mg per adult per meal), putrescine, and cadaverine as toxins

Cite this Article as: Setiani BE, Yunianta, Zubaidah E, Wardani AK and Purwitasari L, 2024. *Lactococcus lactis* spp. *lactis* a promising tool in the control of biogenic amines during soy sauce fermentation. International Journal of Agriculture and Biosciences 14(1): 40-49. https://doi.org/10.47278/journal.ijab/2025.008

A Publication of Unique Scientific Publishers

(Jaguey-Hernández et al., 2021). Although most fermented soybean products in Asia are usually within the limits of biogenic amines safe for human consumption, certain products, such as fermented soybean paste and soy sauce, have been found to contain vasoactive biogenic amines that exceed the toxic threshold (Mah et al., 2019). This is related to the fact that the production of soy sauce, which still applies traditional processes, especially in micro, small, and medium enterprises in Indonesia (Tamang, 2016), tends to be less controlled, so it has a greater potential to contain biogenic amines. Meanwhile, Indonesia does not yet have regulations regarding the content of biogenic amines in soy sauce.

Elevated biogenic amine levels in soy sauce can lead to health problems, including low blood pressure, skin irritation, allergies, migraines, dizziness, high blood pressure, and neurological diseases. Such risks can arise due to the content of histamine, tyramine, putrescine, and cadaverine, in excessive amounts (Ruiz-Capillas & Herrero, 2019). Therefore, maintaining optimal biogenic amine levels in soy sauce is crucial to ensure its safety. Understanding biogenic amine levels and their implications is essential for soy sauce producers and enthusiasts. Various techniques, including chemical and biological methods, have been used to reduce biogenic amine levels in soy sauce (Zhou et al., 2023). Among these methods, the use of microbes, particularly LAB, has gained attention due to their ability to target specific compounds. LAB not only inhibits the activity of biogenic amine-producing organisms but also contributes to the degradation of the biogenic amines produced (Li & Lu, 2020).

Recent research highlights the potential of LAB in reducing biogenic amine levels with studies showing that LAB can effectively reduce biogenic amine concentrations during fermentation (Pištěková et al., 2020; Yilmaz et al., 2022; Qin et al., 2023). Additionally, in the study of Li et al. (2021) LAB has been found to reduce biogenic amines in soybean paste, offering a promising approach that could be applied to soy sauce production, given the similar production steps involving fermented soybeans.

Considering the importance of food safety monitoring for biogenic amine compounds in soy sauce, particularly in Indonesia, it was necessary to evaluate the biogenic amine content and microbiological characteristics in soy sauce produced by small-scale enterprises in Central Java, Indonesia. Remarkably, despite promising findings regarding the role of lactic acid bacteria (LAB) in the reduction of biogenic amines, no prior research has explored their application in soy sauce production. Therefore, this study aimed to address this research gap by isolating LAB from the moromi stages of soy sauce fermentation and evaluating their capacity to reduce biogenic amines during the fermentation process.

MATERIALS & METHODS

Objects and Stages of Fermentation

This study involved twenty samples from five soy sauce brands in Central Java located in Tegal, Pati, Kudus, and Yogyakarta and made by small micro-enterprises in the traditional way coded FA, FB, FD, FD, and FE. The formula is produced using good-quality soybeans in the fermentation process without the addition of wheat flour. The specific proportions of soybeans and salt brine solution were adjusted according to the manufacturer's recipe. The samples consisted of four fermentation stages: koji (coded FX1 as a fermented sample of dried soybeans that had been coated with mold), early moromi (coded FX2 as fermented koji with salt-liquid at the beginning of the fermentation period for a month), final moromi (coded FX3 as fermented koji with salt-liquid that required a fermentation period of several months), and ready-to-eat soy sauce products (coded FX4 as a sweet soy sauce products ready to consume). Biogenic amine standards were purchased from Sigma-Aldrich (USA). Dansyl chloride (derivatizing reagent), L-proline and 1,7-Diaminoheptane (as internal standards) and acetone were from Merck (Germany). HPLC grade acetonitrile C-18 was from Mallinkort (USA). Ultrapure water was generated using a Milli-Q purification system (Millipore, Bedford, MA, USA). The whatman filter paper was from Sigma-Aldirch (USA). Sodium chloride, tryptic soy broth, tryptic soy agar, decarboxylase medium, phosphate buffer, Trichloroacetic acid, sodium hydroxide, n-hexane, butanol, chloroform, acetonitrile, and methanol were from Merck (Germany).

Analysis, Isolation and Identification of lactic acid Bacteria

The assessment of total aerobic and proteolytic bacteria in soy sauce utilized the aerobic plate count and skim milk agar enriched with 3% NaCl. A mixture of 225mL of peptone water containing 0.85% NaCl and 25mL of the sample was homogenized for 2min in a stomacher bag. Subsequently, a 10-fold dilution was performed, and 100µL was taken from each dilution level, spread on agar plates, and incubated (Incubator Memmert, Germany) for 48 hours at 37°C. Bacterial colonies were counted (Colony counter Funke Gerber, Germany) as log colony-forming units (CFU/mL).

Bacterial isolation from the moromi phase of soy sauce included mixing 225mL of trypticase soy broth supplemented with 3% NaCl with 25mL of the sample in a 500mL Erlenmeyer flask, followed by incubation for 24 hours at 37°C in a shaker incubator (Benchmark, US) at 100rpm. The obtained culture was spread on trypticase soy agar plates with 3% NaCl and incubated for 48 hours at 37°C. Colonies with optimal appearance were re-streaked on new trypticase soy agar plates until pure isolates were obtained. Subsequently, the pure isolates underwent analysis for the catalase test and gram staining.

Pure isolates underwent analysis for their ability to perform decarboxylation activity on differential media, where bacteria with amino acid decarboxylase activity change the color of the media around the bacterial colony growth point from yellow to purple (Joosten & Northolt, 1989). Isolates lacking decarboxylase activity were selected for testing their ability to reduce biogenic amine levels. The isolates were identified through a combination of API analysis, PCR, and phylogenetic analysis. The

Analytical Profile Index 50 CHL system (BioMérieux, France) was performed to identify the isolate belonging to the Lactic Acid Bacteria group (Brolazo et al., 2011). Identification of the isolates was carried out using the method outlined by (Stackebrandt & Goebel, 1906). This process involved DNA extraction utilizing the Genomic DNA extraction with Quick-DNA Fungal/Bacterialminiprep Kit (Zymo Research, D6005), followed by DNA amplification using PCR amplification with (2x) My Taq HS Red Mix (Bioline, BIO-25048). The bacterial 16S rRNA gene region was amplified using universal primer sets 27F/1492R. Subsequently, DNA sequencing was performed using Bi-directional Sequencing. Sequence 27F primer is 5'-AGAGTTTGATCMTGGCTCAG-3' while sequence 1492R primer is 5'-TACGGYTACCTTGTTACGACTT-3'. The obtained DNA sequences were then compared with the NCBI database to determine the closest match sequence. Phylogenetic analysis using the Neighbor-Joining (Unrooted Tree) was then subjected to the method via NCBI Blast.

Biogenic Amines Reduction by lactic acid Bacterial Isolates

Evaluation of the reduction of biogenic amine levels by the isolated bacteria followed the protocol outlined by (Leuschner et al.,1998). After an overnight incubation, the bacterial isolates underwent a rinse with 0.05M phosphate buffer (pH 7). The collected pellet was reconstituted in a phosphate buffer solution containing 100ppm of biogenic amines (putrescine, spermidine, spermine, and cadaverine). A 20milliliter aliquot of the suspension, adjusted to a concentration of 10^7CFU/mL, was incubated at 37°C for 24 hours in a shaking incubator (150rpm). Post-incubation, an equal volume of 1M HCl was added to the samples, followed by a boiling step for 10min and subsequent centrifugation (Centrifuge Thermo Scientific, USA) at 9000g for 10min. The resulting supernatant was then frozen at -20°C for subsequent analysis of its biogenic amine levels.

Determination of Biogenic Amines

The analysis of biogenic amine levels comprises several steps, such as initial sample extraction, sample purification, secondary sample extraction, sample derivatization, and standard preparation. The procedures for sample extraction and purification were adapted from methods employed by Cui et al. (2014) and Liu et al. (2020) with certain modifications.

In the first sample extraction, 5% trichloroacetic acid (TCA) was combined with 10mL of the sample. Extraction was carried out for 1 hour, followed by centrifugation at 4000rpm for 10min. The resulting supernatant was diluted with 5% TCA in a 50mL test tube and filtered using filter paper. A 5mL portion of the filtrate was mixed with 1.5g of sodium chloride. Subsequently, 2.5mL of n-hexane was added for 1min using vortex oscillation (Thermo Scientific, USA). The lower layer, formed through n-hexane extraction to eliminate fat content, was separated from the mixture, and the solution's pH was adjusted (pH meter OHAUS, USA) to 12 using a 5M sodium hydroxide solution (Harmayani et al., 2017).

For the second sample extraction, a mixture of butanol and chloroform (1:1) was added to the purified solution for 1min. The mixture was then centrifuged for 5min at 5000rpm. The resulting pellet underwent re-extraction and dilution with the same solution (butanol and chloroform). A 5mL portion of the diluted solution was combined with 1mL of 0.1M hydrochloric acid, followed by evaporation to dryness under nitrogen flow in a water bath at a temperature of 65°C. Sample derivatization. Dissolved each dry extract with 1mL equates. The 0.1mL aliquot was then added with 0.4mL acetonitrile, 0.1mL dansyl chloride, and 0.4mL methanol. The supernatant was discharged then the sediment was dissolved with 0.8mL aqua dest and 0.2mL proline as an internal standard before HPLC analysis (Dičáková et al., 2004).

Data Analysis

This study was carried out with three replications and analyzed using IBM SPSS Statistics software (Version 26, USA). One-way ANOVA was employed, followed by Tukey's Analysis, with a significance level set at $P<0.05$. Descriptive explanations of data in tables and Fig.s, along with standard deviations represented by error bars, are provided. The correlation analysis between total microbes, lactic acid bacteria, and biogenic amines at different stages of soy sauce fermentation was conducted using the Principal Component Analysis (PCA) method, utilizing R Studio software (version 4.3.2, USA) referring to Chmiel et al. (2023) with minor modifications.

RESULTS & DISCUSSION

Biogenic Amines Profile of Soy Sauce at Different Stages of Fermentation Process

The biogenic amine levels during fermentation are shown in Fig. 1a, b, c, and d. Biogenic amine compounds during the fermentation process of natural soy sauce tend to decrease, with a drastic reduction occurring in the final phase or soybean sauce phase. The difference in biogenic amine levels at each stage of fermentation is due to differences in the type and number of microbes in each phase. This finding is in accordance with the opinion of Li et al. (2021), which states that the diversity of types and numbers of microbes at each stage of soybean fermentation results in variations in biogenic amine content. According to research Hu et al. (2023), the reduction of salt content in the moromi phase triggers the proliferation of spoilage bacteria, leading an overproduction of acid and biogenic amines in soy sauce products. Therefore, the natural decrease in biogenic amines during the soy sauce fermentation process, especially at the moromi or brine stage, was suspected to occur due to the fermentation conditions containing high salt content, inhibiting the survival rate of spoilage microbes.

Differences in the soy sauce samples did not affect the spermidine content in the soy sauce. The sample with the lowest average content of the biogenic amine was sample D, both for spermidine and putrescine, and sample B for cadaverine. The difference in biogenic amine content in each sample were suspected to be due to the natural biogenic amine content in the soy sauce raw material,

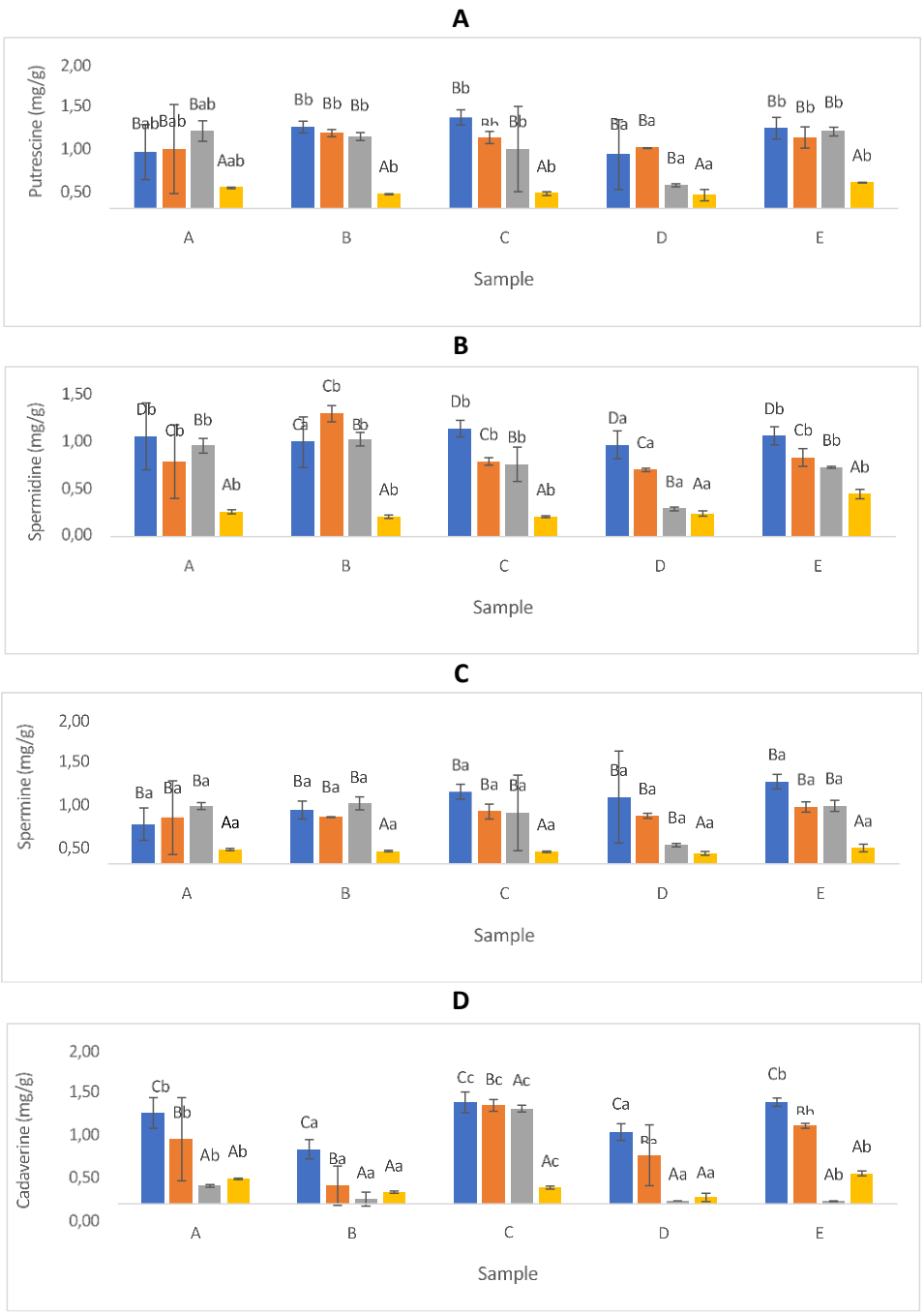

Fig. 1: Putrescine (a), spermidine (b), spermine (c), and cadaverine (d) content of soy sauce at koji (blue), earlier moromi (orange), final moromi (grey), and soybean sauce (yellow) stages of fermentation process. Different letters above the bars indicate statistically significant variations at a significance level of $P < 0,05$ between stages (upper case) and between samples (lower case).

namely soybeans. In research by Oota et al. (2020) and Sagara et al. (2020), it is known that soybeans contain biogenic amine compounds such as spermine and spermidine in germinal and cotyledons tissue, as well as putrescine and cadaverine in the cortex of soybean roots.

The PCA results shown in Fig. 2 explain 95.1% of the variation in the data. Spermine and putrescine were strongly positively correlated, followed by their correlation with spermidine, which also positively correlated with both. However, these three parameters correlated weakly positively with cadaverine, especially weakened with spermine and putrescine. This phenomenon can be attributed to the theoretical understanding that putrescine, spermidine, and spermine are correlated because putrescine is a polyamine compound that is a precursor of spermidine, while spermine is the result of the spermidine synthesis process (Ivanov & Khomutov, 2022; Muñoz-esparza et al., 2021). Consequently, there is no direct relationship between cadaverine and the other three biogenic compounds. In this study, the correlation between spermidine and putrescine, as well as spermine, appears to be slightly weaker compared to the correlation between putrescine and spermine. This is indicated by the formation of a smaller vector angle between putrescine and spermine (Fig. 2), with the average levels of putrescine and spermine being higher than the level of spermidine (Fig. 1a, b, and c for details). This could be interpreted to mean that not all putrescine levels were converted into spermidine, and the spermine level was not only derived from the synthesis of spermidine but also from the raw material, in this case, soybeans.

Zhou et al. (2023) suggested that the content of biogenic amines could be formed through several methods, namely a) decarboxylation of free amino acid

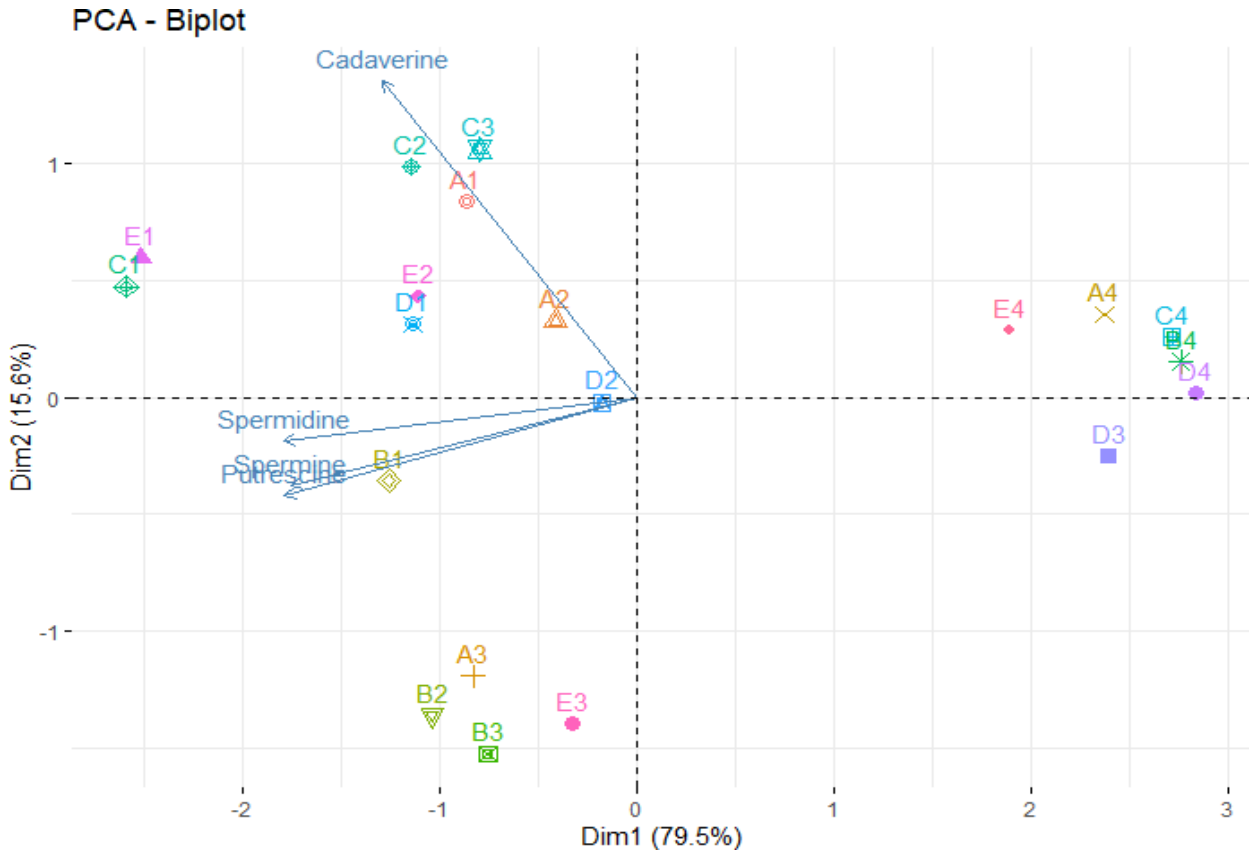

Fig. 2: The correlation relationship between putrescine, spermidine, spermine, cadaverine, and the fermentation stages using Principal Component Analysis (PCA). (1) Koji, (2) Earlier Moromi, (3) Final Moromi, (4) Soybean Sauce. The capital letters denote the five soy sauce samples analyzed in the Fig.s.

compounds contained in the material, but not all of them were converted into biogenic amines, b) formed from transamination of aldehydes and ketones, and c) the addition of yeast rich in free amino acids. Sample D tended to have a relatively low average biogenic amine level (Fig. 1a, b, c, and d). The final phases of moromi and soy sauce showed the lowest average levels of biogenic amines due to their placement at opposing points and a significant distance from the variable vector.

When compared with soy sauce samples in other studies, biogenic amine compounds in soy sauce samples in this study were relatively high. For example, in a study by Li et al. (2020), it was found that the biogenic amine content in the koji phase fermentation of soy sauce was below 40mg/kg and 200mg/kg under open koji fermentation conditions. In another study, Liu et al. (2020) examined the biogenic amine content under high salt fermentation conditions, and the highest level of biogenic amine, spermine, was found to be 402mg/L. Therefore, effective methods to reduce biogenic amines need to be identified.

Total Microbes, Lactic Acid Bacteria and the Correlation of Both Parameters

In this research, an analysis of total microbe count, lactic acid bacteria count, and their correlation at each stage of soy sauce fermentation was conducted. Based on Fig. 3a and b, it was evident that the total microbe count during the soy sauce phase was the lowest compared to other phases, with the highest total microbe count occurring in the koji stage. There was no significant impact of total microbe count on the tasted soy sauce samples. These findings aligned with those of Lai et al. (2022), as their research indicated a decrease in microbe counts with the extended duration of fermentation, especially towards the end of the moromi phase.

In contrast to the earlier explanation regarding the total microbial count, the soy sauce phase had the highest LAB count, while the final moromi phase had the lowest LAB count. Among the soy sauce samples, Sample D was considered to have the lowest average LAB count compared to the other samples. There was no significant difference in LAB count between Samples A, B, C, and E. Ferng et al. (2019) noted that, initially, lactic acid bacteria counts were quite high during the early moromi phase, around 8 log CFU/mL. However, as time passed, during the final moromi phase, the count slightly decreased to approximately 7 log CFU/mL. This trend was similar to the results obtained in this research, but the lactic acid bacteria count increased again in the soy sauce samples ready for consumption. This could be due to inadequate pasteurization and sub-optimal filtration processes. Soy sauce obtained from the final moromi phase undergoes heating or pasteurization to inactivate enzymes and microbes, extending the shelf life of soy sauce (Devanthi & Gkatzionis, 2019). Guo et al. (2019) stated that adding a microfiltration step after pasteurization improved the quality of soy sauce. Their research revealed the absence of live bacteria or yeast in the soy sauce samples that had undergone microfiltration with a membrane.

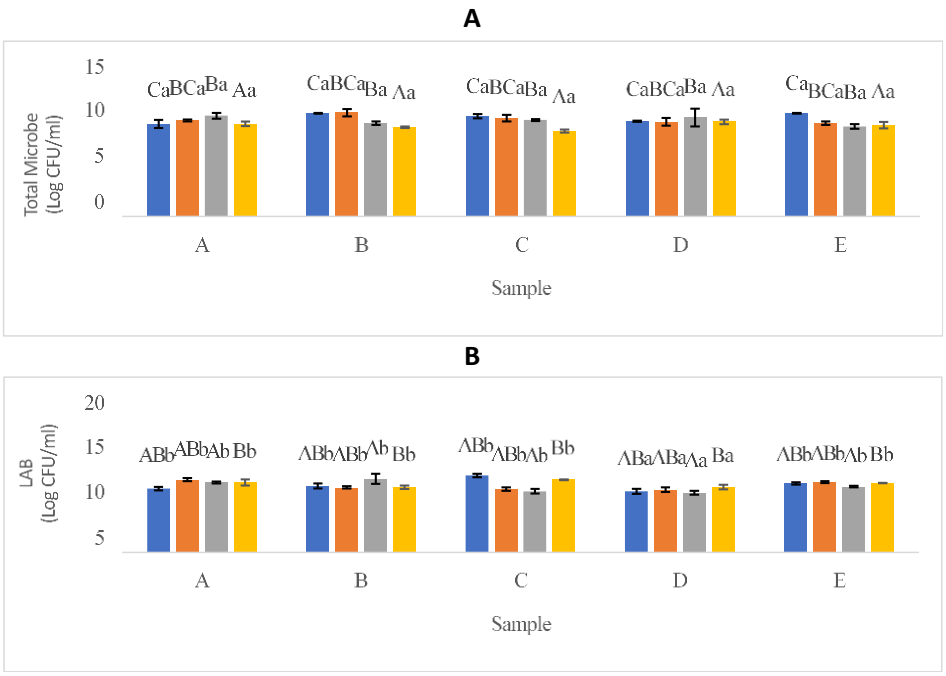

Fig. 3: Total microbe (a) and LAB (b) content of soy sauce at koji (blue), earlier moromi (orange), final moromi (grey), and soybean sauce (yellow) stages of fermentation process. Different letters above the bars indicate statistically significant variations at a significance level of P < 0.05 between stages (upper case) and between samples (lower case).

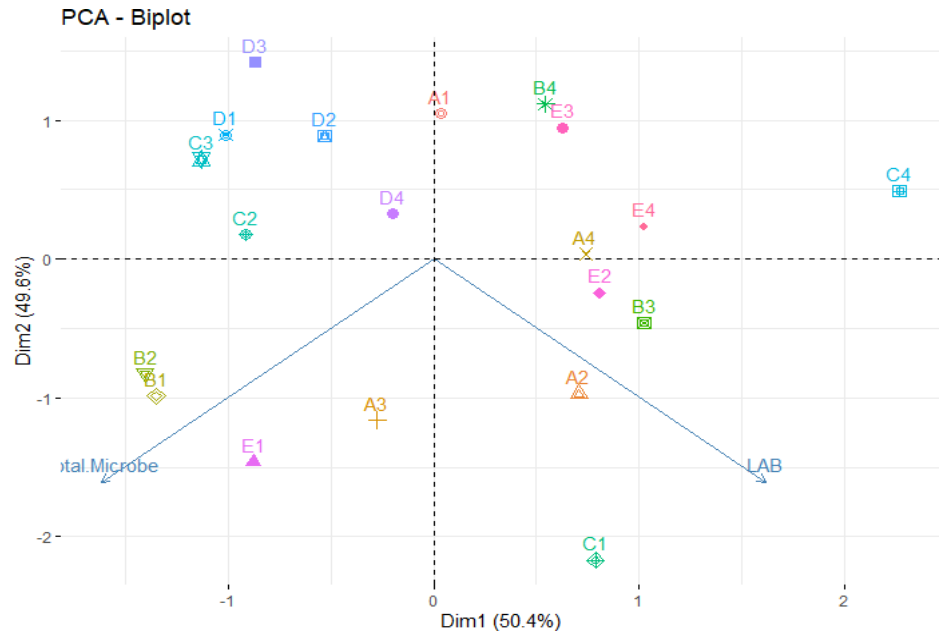

Fig. 4: The correlation relationship between total microbe, lactic acid bacteria, and the fermentation stages using Principal Component Analysis (PCA). (1) Koji, (2) Earlier Moromi, (3) Final Moromi, (4) Soybean Sauce. The capital letters denote the five soy sauce samples analyzed in the Fig.s.

The correlation between the total microbe count and lactic acid bacteria count at each fermentation stage is depicted in Fig. 4, which was a PCA biplot explaining all the data variations, consisting of dim1 (50.4%) and dim2 (49.6%). Based on the angles formed between the total microbe and LAB vectors, it is clear that both have a negative correlation, as they form an angle of more than 90 degrees. This proved that a high total microbe count did not necessarily correlate with a high LAB count, and vice versa. This supported the results shown in Fig. 3a and b, which explained that the soy sauce phase had the lowest total microbe count but a high LAB count, indicating that LAB dominated the total microbial count in soy sauce. Furthermore, from the biplot, it was known that a high total microbe count dominated the koji phase, followed by the early moromi and final moromi phases. Sample C exhibits a high concentration of LAB in both the koji phase and ready-to-consume soy sauce phase, evident from its distant position from other sample points, albeit remaining relatively close to the LAB vector direction. However, the pre-early moromi and final moromi phases exhibit the dominant concentration of LAB. In the fermentation process of soy sauce, fungi such as *Aspergillus oryzae* were used, which could produce proteolytic and amylase enzymes that hydrolyzed soybeans into peptides, amino acids, and simple sugars. These compounds then served as substrates for halophilic bacteria in the moromi phase, resulting in the production of acids such as lactic acid and amino acids that lowered the pH (Ito & Matsuyama, 2021). Therefore, as the fermentation time increased, the microbe count decreased due to the increasingly acidic pH conditions, and the addition of salt in the moromi phase caused many mesophilic bacteria to die, resulting in a predominance of halophilic bacteria in the moromi phase (Khairil et al., 2020). Based on the results obtained, isolation of lactic acid bacteria from the moromi phase was conducted.

Lactic Acid Bacteria Isolation and Degradation of Biogenic Amine by Lactic Acid Bacteria

Based on the results of the spread plate from the soy sauce moromi phase sample, 140 isolates were obtained, which were then subjected to four rounds of a potty quadrant and streak to obtain 8 isolates. These eight isolates underwent morphological form testing, gram staining, catalase analysis, and Moeller decarboxylase analysis, resulting in 2 isolates with morphological form of cocci, gram-positive, and negative results in the catalase and Moeller decarboxylase tests. Biogenic amines are organic compounds produced by microorganisms, especially bacteria such as *Bacillus* and *Staphylococcus*, which possess decarboxylase enzymes. These enzymes selectively remove the carboxyl group (COOH) from precursor amino acids, resulting in biogenic amines and carbon dioxide (Karwowska et al., 2023). The decarboxylase test using Moeller broth media containing yellow-colored amino acid precursors will turn purple after the incubation process if biogenic amines are produced. This change occurs due to the increase in pH resulting from the formation of alkaline compounds (Raje et al., 2019). In this study, the obtained Moeller decarboxylase test results were negative (yellow), indicating that both isolates were unable to perform the decarboxylase reaction and may have the potential to reduce biogenic amines during the soy sauce fermentation process. The two isolates were re-cultured, and 9 colonies were obtained. These colonies were subsequently subjected to testing using the Analytical Profile Index (API 50 CHL kit) to assess their biochemical characteristics and identify their species. Additionally, the colonies were tested for their capacity to reduce biogenic amines.

Based on the API results, it was determined that 9 colonies obtained from the 2 isolates tested were *Lactococcus lactis* bacteria. Several studies have reported that *Lactococcus lactis* has the ability to reduce biogenic amines, as seen in milk (tyramine and histamine) and Dutch cheese (putrescine, spermidine, spermine, cadaverine, and tyramine) (Garbowska et al., 2020; Moghadam et al., 2022). The ability of the sample colonies to reduce biogenic amine compounds is explained in Table 1. Based on the results obtained, it was found that although the nine colonies obtained from 2 isolates are of the same species, namely *Lactococcus lactis*, each colony isolate has different abilities to reduce biogenic amine compounds. The table shows that colonies isolated FC2KB1 and FC2KB3 are capable of reducing all types of biogenic amine compounds, namely putrescine, spermidine, spermine, and cadaverine. However, other colony isolates can only reduce or inhibit the production of some biogenic amine compounds. Bacterial colonies originating from the same isolate may exhibit different characteristics in terms of their ability to inhibit biogenic amines due to factors such as genetic diversification and the environmental conditions of growth. Laroute et al. (2017) revealed that *Lactococcus lactis* is classified into two categories: "domesticated" strains with low diversification and "environmental" strains with high diversification because they originate from natural environments rather than controlled environments. Therefore, it is suspected that *Lactococcus lactis* in this study is an "environmental" strain since it originates from the traditional soy sauce fermentation process. Hernandez-Valdes et al. (2020) added that *Lactococcus lactis* is an amino acid auxotrophic bacterium that is influenced by environmental conditions, meaning it can acquire or lose the ability to synthesize amino acids depending on their availability in the environment.

The biogenic amine compound that all colonies were able to reduce in concentration was cadaverine. This finding aligns with research conducted by Garbowska et al. (2020) and Yilmaz et al. (2022), where *Lactococcus lactis* was found to reduce cadaverine levels in Dutch cheese and lysine decarboxylase broth. The ability of *Lactococcus lactis* to decrease biogenic amine levels in food products, especially fermented products, is due to several factors. Firstly, *Lactococcus lactis* can produce compounds that inhibit the decarboxylation of amino acids into biogenic amines, both directly and indirectly. It has been reported to produce the enzyme α-acetolactate decarboxylase (ALDC), which regulates the biosynthesis of leucine and valine by controlling the flow of acetolactate and acetoin when the accumulation of these two amino acids is too high (Goupil-Feuillerat et al., 1997; Zheng et al., 2022). *Lactococcus lactis* is also known to produce α-ketoisovalerate decarboxylase (Kivd), which can convert amino acids such as leucine and valine into 3-methylbutanal, a non-biogenic amine compound (Schober et al., 2023). Additionally, as a lactic acid bacterium, *Lactococcus lactis* can reduce biogenic amine levels by competing for available nutrients and regulating pH, thereby inhibiting the nutrition intake and growth of biogenic amine-producing bacteria (Barbieri et al., 2019). This is supported by the research of Li et al. (2023), where *Lactococcus lactis* was able to regulate the pH of the Bian-Que Triple-Bean Soup.

PCR and Phylogenetic Analysis

Based on the conducted sequence analysis, it was found that isolates FC2KB1 and FC2KB3 have a similarity of 99.95% and 99.09%, with the *Lactococcus lactis* strain CAU3045. This was further supported by the phylogenetic results depicted in Fig. 5 and 6, where isolate FC2KB1 exhibited 99% kinship with isolate FC2KB3 and the *Lactococcus lactis* strain CAU3045 MF582795.1. Phylogenetics is a field of study within biology that concentrates on comprehending the evolutionary relationships and ancestry among different organisms or species. It entails the analysis of genetic, morphological, and behavioral data to reconstruct evolutionary history, which is represented through a tree-like diagram known as phylogenetic trees or evolutionary trees (Abaza, 2020).

Lactococcus lactis is a Gram-positive, facultatively anaerobic, mesophilic bacterium that is catalase-negative, non-motile, and non-sporulating, it can survive in harsh environments, including the human digestive system (Kazou, 2021; Jeong et al., 2023). *Lactococcus lactis* comprises four species, namely *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *L. lactis* subsp. *hordniae*, and *L. lactis* subsp. *cremoris* (Laroute et al., 2017).

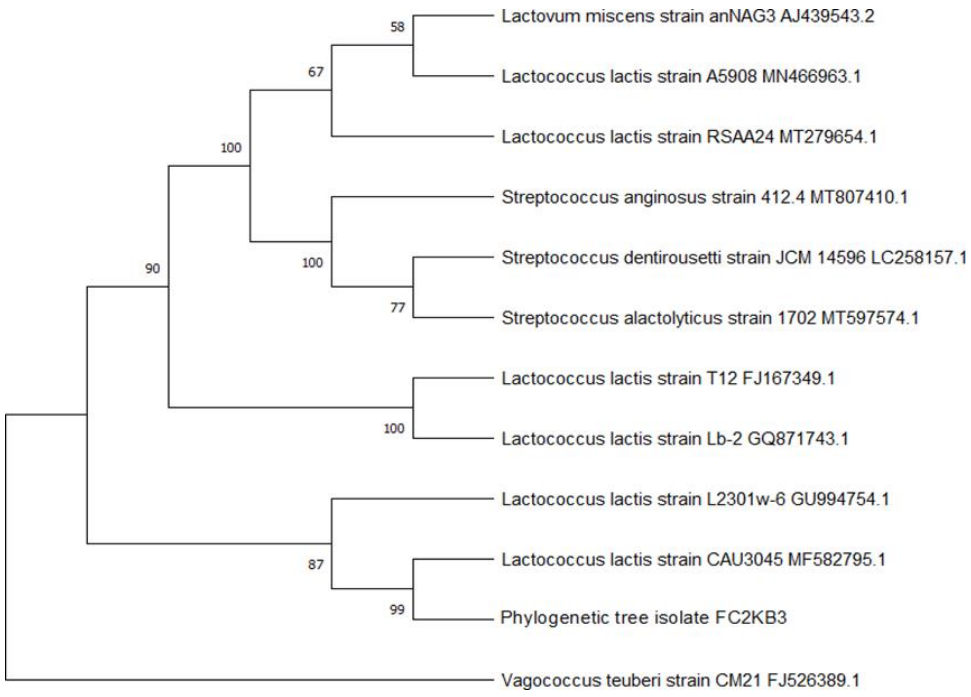

Fig. 5: Phlogenetic of FC2KB1

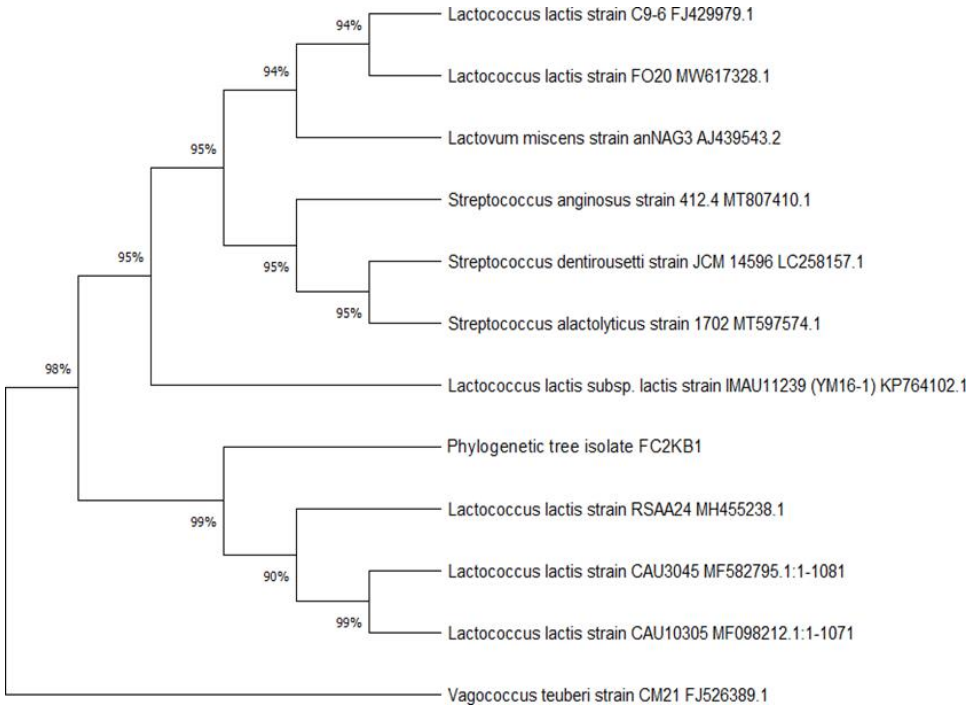

Fig. 6: Phylogenetic of FC2KB3

Classified as generally recognized as safe (GRAS) by the FDA, *Lactococcus lactis* is commonly used in food fermentation, such as in cheese and yogurt production, as well as a food preservative due to its production of acid and bacteriocin, it is also utilized in genetic engineering (Song et al., 2017). While *Lactococcus lactis* is typically isolated from dairy products like milk and kefir grains (Yerlikaya, 2019), this study found it in the fermentation process of soy sauce, suggesting that *Lactococcus lactis* can be found in non-dairy products. This finding was supported by the research of Khalaf & Raizada (2020), who successfully isolated six strains of *Lactococcus lactis* from cucumber seeds, cantaloupe, and acorn squash. Initially occurring in plants, this "environmental" isolate of *Lactococcus lactis* can be transferred to animals and eventually be isolated in dairy products (McAuliffe, 2018). Cavanagh et al. (2015) were also able to isolate *Lactococcus lactis* from grass and corn. Hence, it is suspected that *Lactococcus lactis* in this study emerged possibly due to contact or contamination from the surrounding air closely associated with the presence of plants containing *Lactococcus lactis*.

Conclusion

Based on the conducted research, it can be concluded that traditionally produced soy sauce in Central Java contains relatively high levels of biogenic amines compared to soy sauce from other studies in different countries. The total microbial count in the soy sauce fermentation process decreases as the

fermentation progresses, with the ready-to-consume soy sauce phase exhibiting the lowest total microbial count. However, the higher count of lactic acid bacteria in the ready-to-consume soy sauce might be attributed to inadequate pasteurization and final handling processes. The moromi phase showed the second-highest content of lactic acid bacteria after the ready-to-consume soy sauce phase, which prompted the isolation of lactic acid bacteria during this phase.

Two final isolates, FC2KB1 and FC2KB3, were identified as *Lactococcus lactis* and were found to have a phylogenetic relationship with the *Lactococcus lactis* strain CAU3045. Both isolates demonstrated the capability to degrade four types of biogenic amines: putrescine by 31.28% and 33.76%, spermidine by 16.98% and 18.59%, spermine by 22.5% and 0.1%, and cadaverine by 16.42% and 31.03%. Hence, the isolates FC2KB1 and FC2KB3, obtained from the moromi phase of soy sauce fermentation, have shown promise as a potential alternative method to reduce the biogenic amine content in soy sauce. Further research is required to assess the ability of these two isolates to degrade biogenic amines in the soy sauce fermentation process.

Acknowledgment: This study project was supported by SEAMEO SEARCA (Southeast Asian Regional Center for Graduate Study and Research in Agriculture) with Ref. No. GBG20-3239.

Authors' contribution: Bhakti Etza Setiani: Investigation, analysis, writing – original draft, proofed conception or design, critical revision, final approval of the version to be published. Yunianta: Proofed conception or design, critical revision, final approval of the version to be published. Elok Zubaidah: Proofed conception or design, critical revision, final approval of the version to be published. Agustin Krisna Wardani: Proofed conception or design, critical revision, final approval of the version to be published. Lutfi Purwitasari: Worked on R Studio software (version 4.3.2, USA) for the Principal Component Analysis.

REFERENCES

Abaza, S. (2020). What is and why do we have to know the phylogenetic tree?. *Parasitologists United Journal*, 13(2), 68–71. https://doi.org/10.21608/puj.2020.35843.1082

Barbieri, F., Montanari, C., Gardini, F., & Tabanelli, G. (2019). Biogenic amine production by lactic acid bacteria: A review. *Foods*, 8(1), 1–27. https://doi.org/10.3390/foods8010017

Brolazo, E. M., Leite, D. S., Tiba, M. R., Villarroel, M., Marconi, C., & Simoes, J. A. (2011). Correlation between API 50 CH and multiplex polymerase chain reaction for the identification of vaginal Lactobacilli in isolates. *Brazilian Journal of Microbiology*, 42(1), 225–232. https://doi.org/10.1590/S1517-83822011000100028

Cavanagh, D., Casey, A., Altermann, E., Cotter, P. D., Fitzgerald, G. F., & McAuliffe, O. (2015). Evaluation of *Lactococcus lactis* isolates from nondairy sources with potential dairy applications reveals extensive phenotype-genotype disparity and implications for a revised species. *Applied and Environmental Microbiology*, 81(12), 3961–3972. https://doi.org/10.1128/AEM.04092-14

Chmiel, M., Cegiełka, A., Świder, O., Roszko, M., Hać-Szymańczuk, E., Adamczak, L., Pietrzak, D., Florowski, T., Bryła, M., & Florowska, A. (2023). Effect of high pressure processing on biogenic amines content in skin-packed beef during storage. *Lwt*, 175: 114483. https://doi.org/10.1016/j.lwt.2023.114483

Cui, R. Y., Zheng, J., Wu, C. De, & Zhou, R. Q. (2014). Effect of different halophilic microbial fermentation patterns on the volatile compound profiles and sensory properties of soy sauce moromi. *European Food Research and Technology*, 239(2), 321–331. https://doi.org/10.1007/s00217-014-2225-9

Devanthi, P. V. P., & Gkatzionis, K. (2019). Soy sauce fermentation: Microorganisms, aroma formation, and process modification. *Food Research International*, 364–374. https://doi.org/10.1016/j.foodres.2019.03.010

Dičáková, Z., Bystrický, P., Sokol, J., & Marcinčák, S. (2004). The estimation of biogenic amines in meat by HPLC method. *MESO: Prvi Hrvatski Časopis o Mesu*, 6(6), 38–43. https://hrcak.srce.hr/25896

Ekici, K., & Omer, A. K. (2020). Biogenic amines formation and their importance in fermented foods. *BIO Web of Conferences*, 17, 00232. https://doi.org/10.1051/bioconf/20201700232

Ferng, S., Huang, W.-H., Wu, C.-P., Lu, Y.-T., Hsu, C.-K., Yih-Yuan Chiou, R., & Ting, C.-H. (2019). A controlled fermentation environment for producing quality soya sauce. *Advances in Technology Innovation*, 4(1), 11–20.

Garbowska, M., Pluta, A., & Berthold-Pluta, A. (2020). Impact of nisin-producing strains of *Lactococcus lactis* on the contents of bioactive dipeptides , free amino acids and biogenic amines in dutch type cheese models. *Materials*, 13(8), 1835. https://doi.org/10.3390/ma13081835

Goupil-Feuillerat, N., Cocaign-Bousquet, M., Godon, J. J., Ehrlich, S. D., & Renault, P. (1997). Dual role of α-acetolactate decarboxylase in *Lactococcus lactis* subsp. lactis. *Journal of Bacteriology*, 179(20), 6285–6293. https://doi.org/10.1128/jb.179.20.6285-6293.1997

Guo, H., Huang, J., Zhou, R., Wu, C., & Jin, Y. (2019). Microfiltration of raw soy sauce: Membrane fouling mechanisms and characterization of physicochemical, aroma and shelf-life properties. *RSC Advances*, 9(6), 2928–2940. https://doi.org/10.1039/c8ra08395a

Harmayani, E., Santoso, U., & Gardjito, M. (2017). Indonesian traditional food. Bulaksumur. Yogyakarta: Gadjah Mada University Press: 2017.

Hernandez-Valdes, J. A., van Gestel, J., & Kuipers, O. P. (2020). A riboswitch gives rise to multi-generational phenotypic heterogeneity in an auxotrophic bacterium. *Nature Communications*, 11(1), 1–13. https://doi.org/10.1038/s41467-020-15017-1

Hu, G., Chen, J., Du, G., & Fang, F. (2023). Moromi mash dysbiosis trigged by salt reduction is relevant to quality and aroma changes of soy sauce. *Food Chemistry*, 406: 135064. https://doi.org/10.1016/j.foodchem.2022.135064

Ito, K., & Matsuyama, A. (2021). Koji molds for japanese soy sauce brewing: characteristics and key enzymes. *Journal of Fungi*, 7(8): 658. https://doi.org/10.3390/jof7080658

Ivanov, A. V., & Khomutov, A. R. (2022). Biogenic polyamines and related metabolites. *Biomolecules*, 12(1), 10–14. https://doi.org/10.3390/biom12010014

Jaguey-Hernández, Y., Aguilar-Arteaga, K., Ojeda-Ramirez, D., Añorve-Morga, J., González-Olivares, L. G., & Castañeda-Ovando, A. (2021). Biogenic amines levels in food processing: efforts for their control in foodstuffs. *Food Research International*, 144: 110341. https://doi.org/10.1016/j.foodres.2021.110341

Jeong, H., Kim, S., Hwang, U. S., Choi, H., & Park, Y. S. (2023). Immunostimulatory activity of *Lactococcus lactis* subsp. lactis CAB701 isolated from jeju cabbage. *Microorganisms*, 11(7): 1718. https://doi.org/10.3390/microorganisms11071718

Joosten, H., & Northolt, M. (1989). Detection, growth, and amine producing capacity of Lactobacilli in cheese. *Applied Environment Microbiallogy*, 55(9), 2356–9. https://doi.org/10.1128/aem.55.9.2356-2359.1989

Karwowska, M., Stadnik, J., & Brasca, M. (2023). Aminobiogenic potential of fermented food microbiota. *Frontiers in Microbiology*, 14, 1176149. https://doi.org/10.3389/fmicb.2023.1176149

Khairil, N. A. K., Idris, A., & Hassan, N. (2020). Isolation and identification of halophilic microorganisms in soy sauce. *Food Research*, 4(2), 84–87. https://doi.org/10.26656/fr.2017.4(S2).S04

Khalaf, E. M., & Raizada, M. N. (2020). Draft genome sequences of six strains of *Lactococcus lactis* (phylum firmicutes), spanning the seeds of *Cucumis sativus* L. (cucumber), *Cucumis melo* L. (cantaloupe), and *Cucurbita pepo* var. *turbinate* (acorn squash). *Microbiology Resource Announcment*, 647(8), 8–10. https://doi.org/10.1128/MRA.00665-20

Lai, C. Y., Hou, C. Y., Chuang, P. T., Hsu, W. H., & Wu, S. C. (2022). Microbiota and mycobiota of soy sauce-supplied lactic acid bacteria treated with high pressure. *Fermentation*, 8(7), 1–11. https://doi.org/10.3390/fermentation8070338

Laroute, V., Tormo, H., Couderc, C., Mercier-Bonin, M., Le Bourgeois, P., Cocaign-Bousquet, M., & Daveran-Mingot, M. L. (2017). From genome to phenotype: An integrative approach to evaluate the biodiversity of

Lactococcus lactis. *Microorganisms*, 5(2), 1–17. https://doi.org/10.3390/microorganisms5020027

Leuschner, R.G.K., O'Callaghan, M.J.A., & Arendt, E. K. (1998). Bacilli spoilage in part-baked and rebaked brown soda bread. *Journal of Food Science*, 63(5), 915–8. https://doi.org/10.1111/j.1365-2621.1998.tb17926.x.

Li, B., & Lu, S. (2020). The importance of amine-degrading enzymes on the biogenic amine degradation in fermented foods. *Process Biochemistry*, 99(1), 331–339. https://doi.org/10.1016/j.procbio.2020.09.012

Li, J., Jiang, K., Huang, H., Cheng, H., Ye, X., & Zhi, Z. (2020). Process improvement to prevent the formation of biogenic amines during soy sauce brewing. *Food Chemistry*, *331*(1), 127347. https://doi.org/10.1016/j.foodchem.2020.127347

Li, J., Zhou, L., Feng, W., Cheng, H., Muhammad, A. I., Ye, X., & Zhi, Z. (2019). Comparison of biogenic amines in Chinese commercial soy sauces. *Molecules*, 24(8), 1–10. https://doi.org/10.3390/molecules24081522

Li, S., Du, X., Feng, L., Mu, G., & Tuo, Y. (2021). The microbial community, biogenic amines content of soybean paste, and the degradation of biogenic amines by Lactobacillus plantarum HM24. *Food Science and Nutrition*, 9(12), 6458–6470. https://doi.org/10.1002/fsn3.2528

Li, Y., Yang, H., Yu, B., Wang, J., Zhu, M., Liu, J., Zheng, Z., Qian, Z., Wei, L., Lv, H., Zhang, L., & Xu, Y. (2023). Fermentation improves flavors, bioactive substances, and antioxidant capacity of bian-que triple-bean soup by lactic acid bacteria. *Frontiers in Microbiology*, 14(1), 1–11. https://doi.org/10.3389/fmicb.2023.1152654

Liu, B., Cao, Z., Qin, L., Li, J., Lian, R., & Wang, C. (2020). Investigation of the synthesis of biogenic amines and quality during high-salt liquid-state soy sauce fermentation. *Lwt*, *133*(1), 109835. https://doi.org/10.1016/j.lwt.2020.109835

Mah, J. H., Park, Y. K., Jin, Y. H., Lee, J. H., & Hwang, H. J. (2019). Bacterial production and control of biogenic amines in Asian fermented soybean foods. *Foods*, 8(2), 85. https://doi.org/10.3390/foods8020085

McAuliffe, O. (2018). Symposium review: *Lactococcus lactis* from non-dairy sources: their genetic and metabolic diversity and potential applications in cheese1. *Journal of Dairy Science*, 101(4), 3597–3610. https://doi.org/10.3168/jds.2017-13331

Mills, S., Ross, R. P., & Coffey, A. (2021). Lactic acid bacteria: *Lactococcus lactis*. *Encyclopedia of Dairy Sciences*, 4(1), 218–225. https://doi.org/10.1016/b978-0-12-818766-1.00325-1

Moghadam, M. A. M., Anvar, S. A., Amini, K., & Khani, M. (2022). The effect of *Lactococcus lactis* and bifidobacterium bifidum probiotics cell free supernatants on the expression of HDC and TDC genes in *Staphylococcus* strains isolated from milk. *Food Science and Technology (Brazil)*, 42, 1–7. https://doi.org/10.1590/fst.03221

Muñoz-esparza, N. C., Costa-catala, J., Comas-basté, O., Toro-funes, N., Latorre-moratalla, M. L., Veciana-nogués, M. T., & Vidal-carou, M. C. (2021). Occurrence of polyamines in foods and the influence of cooking processes. *Foods*, 10(8), 1752. https://doi.org/10.3390/foods10081752

Oota, M., Tsai, A. Y. L., Aoki, D., Matsushita, Y., Toyoda, S., Fukushima, K., Saeki, K., Toda, K., Perfus-Barbeoch, L., Favery, B., Ishikawa, H., & Sawa, S. (2020). Identification of naturally occurring polyamines as root-knot nematode attractants. *Molecular Plant*, 13(4), 658–665. https://doi.org/10.1016/j.molp.2019.12.010

Pištěková, H., Jančová, P., Berčíková, L., Buňka, F., Sokolová, I., Šopík, T., Maršálková, K., Amaral, O. M. R. P. de, & Buňková, L. (2020). Application of qPCR for multicopper oxidase gene (MCO) in biogenic amines degradation by *Lactobacillus casei*. *Food Microbiology*, 91, 103550. https://doi.org/10.1016/j.fm.2020.103550

Qin, S., Zeng, X. M., Jiang, M., Rui, X., Li, W., Dong, M. S., Chen, X. H., & Zhang, Q. Q. (2023). Genomic and biogenic amine-reducing characterization of *Lactiplantibacillus planatraum* JB1 isolated from fermented dry sausage. *Food Control*, *154*, 109971. https://doi.org/10.1016/j.foodcont.2023.109971

Raje, U. V. S., Ramlal, S., Batra, H. V., Naika, M., & Jeyabalaji, J. K. (2019). An in-vitro screening for biogenic amines producing microorganisms from fermented foods and its degradation by bacteria from canine saliva. *Journal of Pure and Applied Microbiology*, 13(1), 271–280. https://doi.org/10.22207/JPAM.13.1.29

Ruiz-Capillas, C., & Herrero, A. M. (2019). Impact of biogenic amines on food quality and safety. *Foods*, 8(2), 62. https://doi.org/10.3390/foods8020062

Sagara, T., Bhandari, D. R., Spengler, B., & Vollmann, J. (2020). Spermidine and other functional phytochemicals in soybean seeds: spatial distribution as visualized by mass spectrometry imaging. *Food Science and Nutrition*, 8(1), 675–682. https://doi.org/10.1002/fsn3.1356

Schober, L., Dobiašová, H., Jurkaš, V., Parmeggiani, F., Rudroff, F., & Winkler, M. (2023). Enzymatic reactions towards aldehydes: an overview. *Flavour and Fragrance Journal*, 38(4), 221–242. https://doi.org/10.1002/ffj.3739

Song, A. A. L., In, L. L. A., Lim, S. H. E., & Rahim, R. A. (2017). A review on *Lactococcus lactis*: from food to factory. *Microbial Cell Factories*, 16(1), 1–15. https://doi.org/10.1186/s12934-017-0669-x

Stackebrandt, E., & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology*, 44(4), 869–9. https://doi.org/10.1099/00207713-44-4-846

Surono, S. (2016). Ethnic fermented foods and beverages of Indonesia. In: Tamang JP, editor. Ethnic Fermented Foods and Alcoholic Beverages of Asia. *New Delhi: Springer India*, 341–82. https://doi.org/10.1007/978-81-322-2800-4

Yerlikaya, O. (2019). Probiotic potential and biochemical and technological properties of *Lactococcus lactis* ssp. lactis strains isolated from raw milk and kefir grains. *Journal of Dairy Science*, 102(1), 124–134. https://doi.org/10.3168/jds.2018-14983

Yilmaz, N., Özogul, F., Moradi, M., Fadiloglu, E. E., Šimat, V., & Rocha, J. M. (2022). Reduction of biogenic amines formation by foodborne pathogens using postbiotics in lysine-decarboxylase broth. *Journal of Biotechnology*, 358(1), 118–127. https://doi.org/10.1016/j.jbiotec.2022.09.003

Zheng, Z. Q., Luo, C. Y., Chen, H., Sun, H., Hui, X., Chen, Z. D., Gao, W. Y., & Li, H. (2022). Characterization of acetolactate decarboxylase of *Streptococcus thermophilus* and its stereoselectivity in decarboxylation of α-hydroxy-β-ketoacids. *Bioorganic Chemistry*, 122, 105719. https://doi.org/10.1016/j.bioorg.2022.105719

Zhou, K., Zhang, X., Huang, G. D., Hongsibsong, S., Hao, G., Li, Y. M., Yang, J. Y., & Xu, Z. L. (2023). Formation of biogenic amines in soy sauce and reduction via simple phytochemical addition. *Lwt*, *176*, 114542. https://doi.org/10.1016/j.lwt.2023.114542